

## 5 Patent Claims

1. An assay chip (2) for investigation of the functionality of non-lipid molecules and their interactions with molecules, comprising:

- 10 a) a nanopore substrate (28) having a plurality of nanopores (8);
- b) a suitable substantially planar support layer (6) deposited on said nanopore substrate (28) having a plurality of nanopores (8) corresponding with said  
15 nanopores of said nanopore substrate (28);
- c) a biological effective layer (4) being capable to host at least a non-lipid molecule or functional molecule, deposited on said support layer (6) and covering the plurality of nanopores (8), resulting in accessible  
20 nanopores (8) from both sides of the biological effective layer (4) for measurements.

2. The assay chip (2) according to claim 1, characterized in that

- 25 the surface of the support layer (6) is chemically modified by such as activated hydrophobic or hydrophilic silanes or other components resulting in a support promotion layer (9).

3. The assay chip (2) according to claim 1 or 2, characterized in that

- 30 the suitable support layer (6) is selected from a group containing silicon nitride ( $\text{Si}_3\text{N}_4$ ) or silicon oxide substrate ( $\text{SiO}_2$ ), and the substrate (28) is selected from a group containing silicon and carbon containing materials, polymers,  
35 metals, dielectrics, glass or ceramics.

4. The assay chip according to any one of the preceding claims, characterized in that

the thickness of the substrate and the diameter of the nanopores (8) is chosen in order to result with an aspect ratio in the range of 0.25 to 5, preferably in the range of 0.75 to 2.

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5. The assay chip according to claim 4, characterized in that the diameter of the nanopores (8) is in the range of 50 to 2000 nm, preferably 100 to 2000 nm.

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6. The assay chip according to any one of the preceding claims, characterized in that said nanopores are arranged in a plurality of nanopore array sections (7) having an area in the range of  $1 \times 10^{-6} \text{ mm}^2$  to  $1 \text{ mm}^2$  on the total free standing silicon nitride membrane area (29) of  $1 \times 10^{-6} \text{ mm}^2$  to  $10 \text{ mm}^2$ .

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7. The assay chip according to any one of the preceding claims, characterized in that said nanopores (8) having a distance from each other in the range of 0.5 to 5-times of their diameter, preferably in the range of 0.8 to 2-times of their diameter.

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8. The assay chip according to any one of the preceding claims, characterized in that the biological effective layer is a biomembrane isolated preferably from prokaryotic or eukaryotic cells, or the lipid bilayer is formed by preparation and later fusion of lipid vesicles or is a functional layer of supramolecular assembly.

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9. The assay chip according to any of the preceding claims, characterized in that the non-lipid molecules are from a natural source like cells of eukaryotes or prokaryotes.

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10. The assay chip according to claim 9, characterized in that

the non-lipid molecule is a synthetic compound.

11. The assay chip according to claim 8,  
characterized in that

5 both biomembranes and lipid bilayers comprise at least one  
non-lipid and/or functional molecule (3), whereby the  
functional molecule (3) is produced using recombinant DNA or  
RNA technologies.

10 12. The assay chip according to claim 8,  
characterized in that  
the biological effective layer is made from at least one  
intact living cell.

15 13. A process for analyzing the functionality of a non-lipid  
molecule or functional molecule (3), being integrated in a  
biological effective layer (4) of the assay chip according to  
any one of the preceding claims 1 to 12, comprising:

20 a) applying a fluid containing a binding compound (14, 22)  
to one side of the fluid biological effective layer in  
order to allow the binding compound (14, 22) to interact  
with the non-lipid molecule;

25 b) monitoring the response of the non-lipid molecule  
(3) induced by effector binding (14, 22) and/or the  
interacting of binding molecules (13) in the fluid  
biological effective layer by measuring physical or  
chemical changes on the cis- or trans-side of the assay  
chip (2).

30 14. A use of the assay chip according to any of the preceding  
claims 1 to 12 in a drug discovery process with respect of  
the functionality of membrane proteins (3, 13) in response to  
binding of the potential drugs to be screened binding to the  
membrane protein (3) at the agonist site (15) or allosteric  
35 site (23).

15. The use of the assay chip according to the claims 1 to 14  
for membrane protein preparations to image molecules and  
molecule clusters using microscopic and nanoscopic methods  
(local probe microscopic methods) or measuring light  
5 (especially fluorescence), ions, currents, radioactivity and  
mechanical signals.

16. The use of the assay chip according to the claims 1 to 14  
for applications to investigate or detect macroscopically  
10 molecular processes between two compartments, whereby the two  
compartments with volumes ranging from submilli-liters to  
micro-liters, as part of a (micro)fluid system, are separated  
by the biological effective layer optionally having proteins  
integrated therein, whereby the protein is a transmembrane,  
15 integral, attached through a peptid or to a lipid anchor or  
non covalently adhered to the lipid bilayer (4).